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CUTANEOUS TOXICITY EVALUATION OF AIR FORCE DEVELOPMENT MATERIALS - V

TECHNICAL DOCUMENTARY REPORT No. MRL-TDR-62-26

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Contract Monitor: Alan B. Cooper, Captain, USAF, MC
Project No. 6302, Task No. 630201

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(Prepared under Contract No. AF 33(616)-7931
by Morris V. Shelanski and Karl L. Gabriel
of the
Industrial Biology Research and Testing Laboratories, Inc.,
Philadelphia 4, Pennsylvania)

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FOREWORD

This report was initiated by the Toxic Hazards Section, Physiology Branch, Biomedical Laboratory. The contract monitor was Captain Alan B. Cooper, USAF, MC. The original research and development work upon which the report is based was accomplished by Industrial Biology Research and Testing Laboratories, Inc., 22 N. 36th Street, Philadelphia 4, Pennsylvania, under Air Force Contract No. AF 33(616)-7931, in support of Project No. 6302, "Toxic Hazards of Propellants and Materials," Task No. 630201, "Toxicology." The author, Dr. Morris V. Shelanski, was project director in charge of the basic research and development work. Dr. Karl L. Gabriel was the assistant project director in charge of the work. Research was begun in April 1961 and completed in January 1962. Mr. Louis Shelanski, Animal Physiologist, and Dr. Theodore Levenson, Chemist, cooperated in the research and the preparation of the report.

The animal experimentation reported herein was performed according to the "Rules Regarding Animal Care" as approved by the American Medical Association.

This is the fifth in a series of reports, entitled "Cutaneous Toxicity Evaluation of Air Force Development Materials," by the Industrial Biology Research and Testing Laboratories, Inc. The previous reports are:

- I. WADC TR 56-626, December 1956, by M.V. Shelanski and C. Josephs
- II. WADC TR 57-742 November 1957, by M.V. Shelanski and K.L. Gabriel
- III. WADC TR 59-124, June 1959, by M.V. Shelanski and K.L. Gabriel
- IV. ASD TR 61-77, April 1961, by M.V. Shelanski and K.L. Gabriel

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ABSTRACT

Ten Air Force development materials were studied via the prophetic patch test method on laboratory animals to determine the primary irritant effect, gross sensitization index, and gross percutaneous toxicity of these materials. The patch test studies with rabbits indicated that none of the materials was a primary irritant, sensitizer, nor gross systemic poison. Therefore, there was no contraindication to proceeding with a Shelanski repeated insult patch test on human volunteers. The results of the human patch test indicated that all of the materials were safe to use in contact with human skin.

PUBLICATION REVIEW

This technical documentary report has been reviewed and is approved.

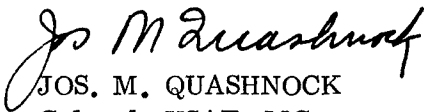

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INTRODUCTION

Industrial Biology Research and Testing Laboratories, Inc., was engaged by the United States Air Force to perform dermatological studies and provide cutaneous toxicity data on certain Air Force development materials. These data would serve the Air Force as criteria for establishing safe handling procedures and limits of application of these materials when utilized by Air Force personnel.

There are various methods used for the determination of cutaneous toxicity of a chemical compound or substance. Laboratory animals, such as rabbits or guinea pigs, have been used by many investigators (ref. 1). The true index of cutaneous reaction can, however, only be determined by using human subjects. Prophetic patch tests are one of the methods used for this purpose (refs. 2 & 3). This test method helps to establish both the primary irritation and sensitization characteristics of a compound brought into contact with the human skin. Prophetic patch test studies were performed on laboratory animals to screen the primary irritant and sensitization characteristics of certain Air Force development materials. The Shelanski repeated insult patch test (ref. 3), in addition to giving information about primary irritation and sensitization characteristics of the compound, will also bring out any fatiguing reactions which may occur on continuous contact of the material with the human skin. This technique was performed on volunteer human subjects to define the characteristics of these compounds on the skin of humans.

MATERIALS

The following materials were received from the 6570th Aerospace Medical Research Laboratories:

1. Epon Resin #826 (Shell)
2. Epoxy Resin #60SC (Maraset)
3. Methyl-Cellosolve (Deicing) (MIL-A8243A) (ref. 4)
4. Bromochloromethane #681D-201-1224
5. Cutting Oil, Lard Oil - C-O-3768 (9150-231-9054)
6. Dy-Chek Penetrant (MIL-I-25135) (ref. 5)
7. Zyglo ZI-2 Penetrant (MIL-I-25135) (ref. 5)
8. Pix-Chek Penetrant (MIL-I-25135) (ref. 5)
9. Spotchek Penetrant SKL-3 (MIL-I-25135) (ref. 5)
10. Zyglo Emulsifier ZE-3 (MIL-I-25135) (ref. 5)

CRITERIA
FOR GRADING PATCH TEST REACTIONS

The investigators have discussed the criteria for grading patch test reactions used by various authors in a previous report, March 1955 (ref. 6). In this study, as in the previous, the following criteria were used by the Industrial Biology Research and Testing Laboratories, Inc.:

- 0 - no reaction, or questionable reaction
- 1+ - definite or clear-cut erythema
- 2+ - marked erythema, greater than present in 1+ reaction
- 3+ - marked erythema, edema, with or without a few vesicles
- 4+ - marked erythema, edema, with vesicles and oozing

RABBIT SCREENING STUDIES

PROCEDURE

Ten groups of five albino rabbits each were used in this study. The animals selected weighed approximately two kilograms each. Prior to use, the animals were placed on colony diet and observed for a period of two weeks. Animals not showing normal weight gain were replaced.

Prior to patching, the fur on the back of each rabbit was closely clipped to expose an area of skin equal to at least 10% of the total body area. This area was then shaved to denude the skin completely. The patch site area was marked with permanent ink to identify the site for later reference.

The test materials were applied to the denuded skin, covered with glassine paper, and held in place by means of a muslin binder. Approximately four grams of each material was spread over the exposed area of skin for each application. Five rabbits per material were used. The first or primary application remained in contact with the denuded skin for forty-eight hours. Upon removal, reactions were graded and recorded. Twenty-four hours after removal of the patches, the sites were examined for delayed reactions.

Following the primary application, the animals were rested for fourteen days. The patch material was then reapplied on the same site as a challenge or sensitization application. Again, after forty-eight hours contact, the patches were removed and reactions graded and recorded. Twenty-four hours later, the sites were examined for delayed reactions.

RESULTS AND CONCLUSIONS

None of the ten materials studied was found to be a primary irritant nor a sensitizer to rabbits. It was therefore considered safe to proceed with the patch testing procedure on human subjects.

HUMAN PATCH TESTS

SHELANSKI REPEATED INSULT PATCH TEST

PROCEDURE

Each material was tested on three hundred human volunteer subjects. The sample was applied with the conventional patch technique to the skin of the subjects for twenty-four hours and then removed. Skin reactions were graded and recorded. The skin was allowed to recuperate for twenty-four hours. This cycle of contact and recuperation was repeated fifteen times for a total of thirty days, the reaction being graded after each application. Following the removal and the grading of the fifteenth application the skin was allowed to recuperate for two weeks. The material was then re-applied on the same subjects for twenty-four hours. Patches were then removed and the reactions were graded and recorded. The first application gave an index of primary irritation. The final application gave information on sensitization. The repeated applications, from the second through the fifteenth, determined the extent of fatiguing and served to accelerate skin reactions which facilitated forecasting of probability of cutaneous irritation due to long-term exposures.

RESULTS

Material #1 - Epon Resin #826 (Shell) - was not a primary irritant on the skin of laboratory animals or the three hundred human volunteer subjects. It did produce fatiguing action on the skin of 9 subjects. The intensity of this fatiguing action was equivalent to or less than that which may be obtained from 10% weight/volume aqueous solutions of many commercially available toilet soap bars when tested under the same conditions. This material did not sensitize any of the three hundred subjects.

Material #2 - Epoxy Resin #60SC (Maraset) - was not a primary irritant on the skin of laboratory animals or the three hundred human volunteer subjects. It did produce fatiguing action on the skin of 24 subjects. The intensity of this fatiguing action was equivalent to or less than that which may be obtained from 10% weight/volume aqueous solutions of many commercially available toilet soap bars when tested under the same conditions. This material did not sensitize any of the three hundred subjects.

Material #3 - Methyl-Cellosolve (Deicing) (MIL-A8243A) - was not a primary irritant on the skin of laboratory animals or the three hundred human volunteer subjects. It did produce fatiguing action on the skin of 32 subjects. The intensity of this fatiguing action was equivalent to or less than that which may be obtained from 10% weight/volume aqueous solutions of many commercially available toilet soap bars when tested under the same conditions. This material did not sensitize any of the three hundred subjects.

Material #4 - Bromochloromethane #681D-201-1224 - was not a primary irritant on the skin of laboratory animals and was not a primary irritant or a fatiguing agent to the three hundred human volunteer subjects. This material did not sensitize any of the three hundred subjects.

Material #5 - Cutting Oil, Lard Oil - C-O-3768 (9150-231-9054) - was not a primary irritant on the skin of laboratory animals and was not a primary irritant or a fatiguing agent to the three hundred human volunteer subjects. This material did not sensitize any of the three hundred subjects.

Material #6 - Dy-Chek Penetrant (MIL-I-25135) - was not a primary irritant on the skin of laboratory animals and was not a primary irritant or a fatiguing agent to the three hundred human volunteer subjects. This material did not sensitize any of the three hundred subjects.

Material #7 - Zyglo ZI-2 Penetrant (MIL-I-25135) - was not a primary irritant on the skin of laboratory animals and was not a primary irritant or a fatiguing agent to the three hundred human volunteer subjects. This material did not sensitize any of the three hundred subjects.

Material #8 - Pix-Chek Penetrant (MIL-I-25135) - was not a primary irritant on the skin of laboratory animals and was not a primary irritant or a fatiguing agent to the three hundred human volunteer subjects. This material did not sensitize any of the three hundred subjects.

Material #9 - Spotchek Penetrant SKL-3 (MIL-I-25135) - was not a primary irritant on the skin of laboratory animals and was not a primary irritant or a fatiguing agent to the three hundred human volunteer subjects. This material did not sensitize any of the three hundred subjects.

Material #10 - Zyglo Emulsifier ZE-3 (MIL-I-25135) - The intensity of the reactions involved in this fatiguing action was less than or equivalent to that which may be obtained from a 10% weight/volume solution of many commercially available toilet soap bars when tested under the same conditions. However, the number of reactions and the number of subjects involved in such reactions were excessive when judged by the same criterion. In respect to sensitization, in using three hundred subjects for these tests, we can state with 95% statistical certainty that the material tested will be a sensitizer to less than 1.2% of the consumer population inasmuch as none of the three hundred subjects showed any sensitizing reactions (ref. 7).

CONCLUSIONS

In this study ten materials produced no significant reactions by either the Schwartz prophetic patch test on rabbits (ref. 2) or the Shelanski repeated insult patch test on three hundred human volunteers (ref. 3). These materials may be considered innocuous and may be permitted to contact human skin for prolonged periods.

While all the interpretations and recommendations have been made on the basis of a generally accepted testing procedure, it must be pointed out that the test method is not infallible or above criticism. Further, the patch test situation does not duplicate the range of temperature, humidity, air flow, perspiration, and friction, among other factors, which will be met in actual usage of the material. Because the prophetic patch test was devised to provide screening information with respect to cutaneous irritation and sensitivity from certain materials, it must be emphasized that the test should be used only for that purpose. Therefore, the recommended procedure following the test is to employ the materials within the limits recommended for direct skin contact on a usage basis. This should be done on 5,000 to 10,000 subjects, preferably under variable climatic conditions prior to the release of the materials for general use.

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APPENDIX

REPEATED INSULT PATCH TEST

WITH

EPON RESIN #826 (SHELL)

RESULTS

Maximum Intensity Of Reactions

Number of subjects negative throughout	291
Number of subjects showing 1+ but no higher	9

Grade Of Reaction

Number of Application	0	1+	2+	3+	4+
1	300	0	0	0	0
2	300	0	0	0	0
3	300	0	0	0	0
4	300	0	0	0	0
5	300	0	0	0	0
6	300	0	0	0	0
7	300	0	0	0	0
8	300	0	0	0	0
9	295	5	0	0	0
10	294	6	0	0	0
11	297	3	0	0	0
12	295	5	0	0	0
13	300	0	0	0	0
14	298	2	0	0	0
15	294	6	0	0	0
Sub Total	4,473	27	0	0	0
Challenge	300	0	0	0	0
TOTAL	4,773	27	0	0	0

REPEATED INSULT PATCH TEST

WITH

EPOXY RESIN #60SC (MARASET)

RESULTS

Maximum Intensity Of Reactions

Number of subjects negative throughout	276
Number of subjects showing 1+ but no higher	22
Number of subjects showing 2+ but no higher	2

Grade Of Reaction

Number of Application	0	1+	2+	3+	4+
1	300	0	0	0	0
2	299	1	0	0	0
3	300	0	0	0	0
4	300	0	0	0	0
5	300	0	0	0	0
6	296	4	0	0	0
7	300	0	0	0	0
8	300	0	0	0	0
9	294	6	0	0	0
10	291	8	1	0	0
11	283	17	0	0	0
12	285	14	1	0	0
13	289	10	1	0	0
14	290	10	0	0	0
15	291	8	1	0	0
Sub Total	4,418	78	4	0	0
Challenge	300	0	0	0	0
TOTAL	4,718	78	4	0	0

REPEATED INSULT PATCH TEST

WITH

METHYL-CELLOSOLVE (DEICING) (MIL-A8243A)

RESULTS

Maximum Intensity Of Reactions

Number of subjects negative throughout	268
Number of subjects showing 1+ but no higher	28
Number of subjects showing 2+ but no higher	4

Grade Of Reaction

Number of Application	0	1+	2+	3+	4+
1	300	0	0	0	0
2	298	2	0	0	0
3	295	5	0	0	0
4	294	6	0	0	0
5	294	6	0	0	0
6	290	10	0	0	0
7	286	14	0	0	0
8	285	15	0	0	0
9	288	12	0	0	0
10	290	10	0	0	0
11	285	15	0	0	0
12	284	16	0	0	0
13	282	18	0	0	0
14	279	19	2	0	0
15	273	23	4	0	0
Sub Total	4,323	171	6	0	0
Challenge	300	0	0	0	0
TOTAL	4,623	171	6	0	0

REPEATED INSULT PATCH TEST

WITH

ZYGLO EMULSIFIER ZE-3 (MIL-I-25135)

RESULTS

Maximum Intensity Of Reactions

Number of subjects negative throughout	174
Number of subjects showing 1+ but no higher	126

Grade Of Reaction

Number of Application	0	1+	2+	3+	4+
1	300	0	0	0	0
2	246	54	0	0	0
3	231	69	0	0	0
4	240	60	0	0	0
5	233	67	0	0	0
6	243	57	0	0	0
7	226	74	0	0	0
8	209	91	0	0	0
9	186	114	0	0	0
10	209	91	0	0	0
11	203	97	0	0	0
12	196	104	0	0	0
13	206	94	0	0	0
14	208	92	0	0	0
15	202	98	0	0	0
Sub Total	3,338	1,162	0	0	0
Challenge	300	0	0	0	0
TOTAL	3,638	1,162	0	0	0

Aerospace Medical Division,
6570th Aerospace Medical Research
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2. Materials
3. Rabbits
4. Man
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